PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
DEETH WILLIAMS WALL LLP
400 - 150 York Street
TORONTO, Ontario
Canada, M5H 3S5

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 8 September 2006 (08-09-2006)

Applicant's or agent's file reference
30919-728001

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2004/004472	29 October 2004 (29-10-2004)	31 October 2003 (31-10-2003)

International Patent Classification (IPC) or both national classification and IPC
IPC: *A61K 48/00* (2006.01), *A61K 8/64* (2006.01), *A61K 31/455* (2006.01), *A61K 31/70* (2006.01), *A61K 38/17* (2006.01), *A61K 38/18* (2006.01), *A61K 8/49* (2006.01), *A61P 17/00* (2006.01), *A61P 35/00* (2006.01), *A61P 43/00* (2006.01), *A61P 9/00* (2006.01), *A61Q 19/00* (2006.01), *A61Q 19/08* (2006.01)

Applicant
ROBARTS RESEARCH INSTITUTE ET AL

1. This opinion contains indications relating to the following items :

[X]	Box No. I	Basis of the opinion
[X]	Box No. II	Priority
[X]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[X]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[X]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**
If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/CA	Date of completion of this opinion	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	3 August 2006 (03-08-2006)	Debora Fujimoto (819) 997-1855

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

[X] the international application in the language in which it was filed

[ ] a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

[ ] a sequence listing

[ ] table(s) related to the sequence listing

b. format of material

[ ] on paper

[ ] in electronic form

c. time of filing/furnishing

[ ] contained in the international application as filed.

[ ] filed together with the international application in electronic form

[ ] furnished subsequently to this Authority for the purposes of search.

3 [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statement that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments :

Box No. II Priority

1. [X] The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.

2. [ ] This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary :

The priority claim based on US 60/573,339, filed 25.04.2004, cannot be verified as it was unavailable to this International Search Authority at the establishment of the Written Opinion.

Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of :

[ ] the entire international application

[X] claim Nos. 1-28

because:

[X] the said international application, or the said claim Nos. 1-28 relate to the following subject matter which does not require an international search *(specify)* :

Although claims 1-28 encompass a method of treatment of the human/animal body which this Authority is not required to examine under Rule 67.1(iv) of the PCT, the Written Opinion has been established on the basis of the alleged effects of the compounds referred to therein.

[ ] the description, claims or drawings *(indicate particular elements below)* or said claim Nos. are so unclear that no meaningful opinion could be formed *(specify)* :

[ ] the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify):*

[ ] no international search report has been established for said claims Nos.

[ ] a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

[ ] furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

[ ] furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

[ ] pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

[ ] a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

[ ] the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

[ ] See Supplemental Box for further details.

Box No. IV Lack of unity of invention

1. [ ] In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit :

   [ ] paid additional fees

   [ ] paid additional fees under protest and, where applicable, the protest fee

   [ ] paid additional fees under protest but the applicable protest fee was not paid

   [ ] not paid additional fees

2. [X] This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   [ ] complied with

   [X] not complied with for the following reasons :

   This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under Rule 13.1 of the Regulations under the PCT:

   Group I - Claims 1-13, 20-29, 32, 33, 36, and 37 are directed to a method and composition comprising pre-B cell colony-enhancing factor (PBEF) that results in modulation of the intracellular concentration of nicotinamide adenine dinucleotide (NAD); and
   Group II- Claims 38, 41, 42, 45, and 46 are directed to a method and composition comprising phosphoribosyl pyrophosphate (PRPP) that results in modulation of the intracellular concentration of nicotinamide adenine dinucleotide (NAD),
   wherein claims 14-19 and 30, 31, 34, 35, 39, 40, 43, and 44 can belong in either **Group I** or **Group II**.

   Since the nucleotide and amino acid sequences of pre-B cell colony-enhancing factor (PBEF) and phosphoribosyl pyrophosphate (PRPP) and their roles in the modulation of nicotinamide adenine dinucleotide (NAD+) via the NAD+ salvage pathway are known, the claims of **Groups I** and **II**, directed to methods and compositions comprising these known proteins, lack a common special technical feature.

4. Consequently, this opinion has been established in respect of the following parts of the international application :

   [X] all parts

   [ ] the parts relating to claim Nos.

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-46	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-46	NO
Industrial applicability (IA)	Claims	1-46	YES
	Claims	none	NO

2. Citations and explanations :

D1 RONGVAUX A et al. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. EUR J IMMUNOL 2002 Vol 32, pages 3225-3234
D2 RONGVAUX A et al. Reconstructing eukaryotic NAD metabolism. BIOESSAYS Jul 2003 Vol 25, No 7, pages 683-690
D3 ANDERSON RM et al. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. BIOL CHEM 24 May 2002 Vol 277, No 21, pages 18881-18890
D4 BITTERMAN KJ et al. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. J BIOL CHEM 22 Nov 2002 Vol 277, No 47, pages 45099-45107

The problem to be solved is the provision of a composition comprising at least one of (i) pre-B-cell colony-enhancing factor (PBEF) or (ii) phosphoribosyl pyrophosphate (PRPP) and optionally, forms of nicotinamide, and use thereof to treat diseases or conditions in an animal by modulation of the intracellular concentration of nicotinamide adenine dinucleotide (NAD+).

D1 discloses that the murine pre-B-cell colony-enhancing factor (PBEF) is a nicotinamide phosphoribosyltransferase, the first enzyme in the salvage pathway allowing recycling of nicotinamide to nicotinamide adenine dinucleotide (NAD+), and found in all tissues examined (abstract; pages 3226, 3228; Figure 1). D1 also discloses that PBEF is up-regulated in activated T lymphocytes. Therefore, D1 discloses that PBEF modulates cellular metabolism via the NAD+ salvage pathway.

D2 discloses the conversion of nicotinamide to nicotinamide adenine dinucleotide (NAD+) occurs via a nicotinamide salvage pathway in which nicotinamide phosphoribosyltransferase catalyzes the condensation of nicotinamide with phosphoribosyl pyrophosphate (PRPP) (pages 687-688). Additionally, D2 discloses that the human homologue of nicotinamide phosphoribosyltransferase, i.e., pre-B-cell colony-enhancing factor (PBEF), is expressed at high levels in tumor cells, suggesting that increased NAD+ turnover confers a selective growth advantage to tumor cells (page 689).

D3 discloses increasing the level of nicotinate phosphoribosyltransferase (*NPT1*), an enzyme in the NAD+ salvage pathway, creates increased flux in said pathway, thereby increasing Sir2-dependent silencing and extending yeast cell life span (pages 18881-18882). D3 further discloses that an increased cell life span is facilitated by the increased flux of NAD+ through the NAD+ salvage pathway that results in modulation of Sir2. Therefore, D3 discloses that components of the NAD+ salvage pathway are targets to regulate cell longevity (page 18889).

D4 discloses that nicotinamide is an inhibitor of Sir2. D4 further discloses that increased copy number of Sir2 suppresses recombination and extends yeast life span. Similarly, an increased dosage of Sir2 homologue extends the life span of *Caenorhabditis elegans*, and an increased dosage of the human homologue SIRT1 inhibits apoptosis, thereby increasing cell longevity (abstract; pages 45099-45101).

Novelty:
None of D1-D4 taken independently specifically disclose the compositions comprising at least one of either pre-B-cell colony-enhancing factor (PBEF) or phosphoribosyl pyrophosphate (PRPP), and optionally, nicotinamide, and methods thereof to modulate NAD+ via the NAD+ salvage pathway for the treatment of diseases and conditions in an animal. Therefore the subject matter of claims 1-46 is novel in view of any one of D1-D4 taken independently and complies with Article 33(2) of the PCT.

(Continued in Supplemental Box)

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted :

Description Defects:
Paragraph [00132] is redundant in view of paragraph [0068].

Drawing Defects:
Figure 6 does not comply with Rule 11.13(a) of the PCT. Drawings should be executed in durable, black, sufficiently dense, uniformly and well-defined lines. Therefore, the lines depicting the tree in said figure should be amended to comply with Rule 11.13(a).

Figure 9 does not comply with Rule 11.13(e) of the PCT. All numbers and letters should be clear. However, the lettering for (A) through (I) are not clearly visible.

Figure 12 does not comply with Rules 11.11(a) and 11.13(e) of the PCT. The drawings should not contain text matter, except a single word or words, when absolutely indispensable, or a few short catchwords indispensable for understanding. Additionally, said figure legend refers to "Figure 1". The figure legend should be removed to comply with Rule 11.11(a). Further, to comply with Rule 11.13(e), all numbers and letters should be clear. The drawing on page 12/16 is labelled "FIGURES 12(A) - 12(C)"; however, no "(A)", "(B)" or "(C)" designations are present in the figure.

Figures 13 and 14 do not comply with Rule 11.13(e) of the PCT. All numbers and letters should be clear. However, the panels in said figures are not clearly identified as "A" and "B", respectively.

Figures 15 and 16 do not comply with Rules 11.11(a) and 11.13(a) of the PCT. To comply with Rule 11.11(a), the drawings should not contain text matter, except a single word or words, when absolutely indispensable, or a few short catchwords indispensable for understanding. The text at the bottom of said figures should be removed. To comply with Rule 11.13(a), drawings should be executed in durable, black, sufficiently dense, uniformly and well-defined lines. Therefore, the arrows and boxes of said Figures 15 and 16 should be amended.

Claim Defects:
Claim 2 contains a typographical error in the phrase "animal a sufficient amount of PBEF".

Claims 4, 13, 25, 35 and 44 contain two instances each of "topical" and "intraperitoneal" administration, resulting in redundancy within the claim.

Claim 20 should be amended to end with a period.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made :

Description Defects:
The description does not comply with Article 5 of the PCT. In paragraph [0004], the document of Revollo et al should be identified as available online as DOI:10.1074/jbc.M408388200, as the Sept. 20, 2004 online publication date is disclosed in the present application, and the references of Song et al and Hasmann et al are not fully identified. Further, it is unclear what the phrase "INSERT re Amgen/Samal PBEF" in paragraph [0004] means. Additionally, there are citations that are not fully identified in the following paragraphs: [0064] to [0066], [0068], [0069], [0071] to [0074], [0078], [0079], [00102] to [00104], [00106] to [00110], [00129], [00132], and [00133].

The description does not comply with Article 6 of the PCT. A statement, such as found in paragraphs [0009], [0062], and [00134], which implies that the protection sought may be expanded to cover the "spirit" of the invention, should be removed.

Claim Defects:
Claim 1 does not comply with Rule 6.3 (a) of the PCT. As presently drafted, claim 1 comprises a step that consists of a desired result, i.e., "optimizing the intracellular concentration of PBEF in the cells", and not the technical features that are required to achieve that result. The claim must explicitly state the technical features for which protection is being sought.

Claim 6 does not comply with Article 6 of the PCT. It is unclear how viral vectors can be a "non viral plasmid vectors". Further, there is no antecedent for "one or more viral vectors" in claim 3, upon which claim 6 is dependent. It appears that claim 6 should depend on claim 5.

Claim 7 does not comply with Article 6 of the PCT. There is no antecedent for "increasing of said PBEF" in claim 3, upon which claim 7 is dependent. It appears that claim 7 should depend on claim 2.

Claims 8, 9 and 10 do not comply with Article 6 of the PCT. It is unclear what product or process is claimed by the use of the vague and broad phrase "by up (down)-regulating the nucleic acid process which support (increase/repress) the ... production of PBEF".

Claim 13 does not comply with Article 6 of the PCT. There is no antecedent for "administering of said modulator" in claim 11, upon which claim 13 is dependent. It appears that claim 13 should depend on claim 12.

Claims 16 and 17 do not comply with Article 6 of the PCT. It is unclear what product or process is claimed by the vague and broad phrase "by up (down)-regulating the nucleic acid processes which increase (repress) the production of PRPP". Further, there is no antecedent for "promotion of said intracellular production of PBEF" in claim 14, upon which claims 16 and 17 are dependent. It appears that claims 16 and 17 should depend on claim 15.

Claim 22 does not comply with Article 6 of the PCT. There is no antecedent for "said severe stress" in claim 1, upon which claim 22 is dependent. It appears that claim 22 should depend on claim 21.

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of: **Box No. V**

Inventive Step:
D1 or D2 each independently discloses that pre-B-cell colony-enhancing factor (PBEF) has a known role and function in the NAD+ salvage pathway, and that modulation of PBEF alters the NAD+ concentration and flux in different cell types and tissues. D2 additionally discloses the roles of PBEF, nicotinamide and phosphoribosyl pyrophosphate (PRPP) in said pathway. However, a skilled artisan would recognize that a modification of the level or activity of any enzyme or the level of any precursor in said pathway would result in modulation of NAD+ in the cell or tissue of an animal, and that said NAD+ modulation can be used to treat a disease or condition *in vivo*. D3 or D4 each independently discloses that targeting components of the NAD+ salvage pathway for modulation increases cell longevity and decreases apoptosis. D3 or D4 do not specifically disclose targeting PBEF, PRPP, or nicotinamide for modulation to treat diseases and conditions in an animal. However, in view of D1 or D2, in combination with either D3 or D4, which disclose the effect of modulation of Sir 2 via NAD+ flux to increase cellular longevity, a skilled artisan would recognize that any component of the NAD+ salvage pathway is a viable target for modulation of NAD+ via the NAD+ salvage pathway to treat a disease or condition and to increase cellular longevity. Additionally, standard modes of delivery, administration and carriers of pharmaceutical or cosmetic compositions are well known in the art and as such, do not constitute an inventive technical feature. The subject matter of claims 1-46 lacks an inventive step in view of either D1 or D2 independently, taken together with either of D3 or D4 independently, and does not comply with Article 33(3) of the PCT.

Industrial Applicability:
For the assessment of claims 1-28 on the question of whether or not they define subject matter that has industrial applicability, no unified criteria exists in the PCT. Further, the patentability of said claims can depend upon their formulations. The methods *per se* defined in claims 1-28 relate to subject matter which this Authority is not obliged to examine under Rule 67.1(iv) of the PCT, but the alleged effects of the combinations referred to therein for use in the methods appear to represent subject matter that has industrial applicability under Article 33(4) of the PCT.

The subject matter of claims 29-46 appears to have industrial applicability under Article 33(4) of the PCT.